PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 443294GA	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/004125	International filing date *(day/month/year)* 19.04.2004	Priority date *(day/month/year)* 25.04.2003
International Patent Classification (IPC) or national classification and IPC B01L3/00, C12Q1/68, G01N1/28		
Applicant NOVEMBER AKTIENGESELLSCHAFT GESELLSCHAFT F]R ...		

1. This report is the International preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (Indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.11.2004	27.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Hocquet, A Telephone No. +31 70 340-2928

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/004125

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-60	as originally filed
Claims, Numbers	
1-53	received on 10.09.2005 with letter of 09.09.2005
Drawings, Sheets	
1/7-7/7	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	33-53
	No:	Claims	1-32
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-53
Industrial applicability (IA)	Yes:	Claims	1-53
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

The following documents are referred to in this report:

D1 : US 5 229 297 a (SEABERG LEONARD J ET AL) 20 July 1993
D2 : US 4 889 692 a (HOLTZMAN MARC E) 26 December 1989
D3 : US 2002/018998 A1 (STEINBISS JOACHIM ET AL) 14 February 2002
D4 : US 4 585 623 a (CHANDLER HOWARD M) 29 April 1986
D5 : WO 03/031275 a (SIGNATURE BIOSCIENCE INC) 17 April 2003
D6 : US 6 197 595 B1 (FODOR STEPHEN P a ET AL) 6 March 2001

Re Item V

1 When compared to any of the documents D1, the subject-matter of claim 1 is not new (Article 33(2) PCT), because D1 discloses in combination all the features defined in that claim:
Document D1 discloses devices with chambers with pistons (figures 9-14) for reversibly changing their volumes connected by channels and with a sealable inlet port 22 (see c 13, l 63-69) which constitutes a connector with flow regulating means connected to a chamber (eg figure 14). The channel themselves can be provided with flow regulating means in form of pinch valves P1-P5 (c 12 , l 19-35). D1 mentions that a chamber connected to the channels can be filled with a reagent sealed in form of prepackaged ampules (c 15, l 63-66).

2 The subject-matter of claim 1 does not involve an inventive step in the sense of Article 33(3) PCT over document D2:

2.1 Document D2 discloses chambers A-F with pistons for reversibly changing their volume, the chambers being connected via channels 36 in a connector which acts as a rotating valve regulating the flow between the chambers. D2 does not disclose the incorporation of binding material in the chambers. The purpose of the device described in D2 is to prepare samples of samples and avoid contamination during this preparation. Two examples of specific preparation are given (filtration by a filter contained in one of the chamber) or liquid-liquid extraction, but it is clear to the reader that the structure of D2 (piston chambers connected by valve channels) is not limited to any specific mechanical or chemical treatment of the sample (D2, c 4, l 43-45), provided this chemical or mechanical treatment can take place in a piston chamber. The device of D2 contains at least 3 chambers and the chambers are connectable to

the channels according to the position of the valves (see figures 2,4,7)

5.1 Claim 1 differs from D2 by the provision in one of the chamber of a reactant prior to use. But providing a prepackaged sealed reactant in one of the chamber is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to bring the required reactants to the device.

2.1 When compared to D1, the subject-matter of claim 32 is not new (Article 33(2) PCT), because D1 discloses a device with a base plate with channels (figure 14) and a detachable chamber in form of a pre-packaged sealed ampule filled with reagents (c 15, l 63-66) is connectable (by insertion in a compartment) to the channel.

2.2 When compared to D4, the subject-matter of claim 32 is not new in the sense of Article 33(2) PCT, because D4 discloses a baseplate with a channel 20 (see figure 2) provided with one way valves 30. A chamber (syringe 40 in figure 1) filled with a reactant (sample) and sealed (sample retained by capillarity in the space between its end and the notch 42 and sealed by plunger 43) is connectable to the channel before to use (figure 1).

3 As long as it relates to the use of a known device, the subject-matter of claim 33 does not involve an inventive step in the sense of Article 33(3) PCT because the step of moving back and forth a sample solution so that it contacts and binds to a material is known either from D3 (paragraphs 59 and 60) or from D6 (from column 25, line 63).

4 The subject-matter of dependent claims 2-31 or 34-53 does not involve an inventive step in the sense of Article 33(3) PCT, because their features have already been employed for the same purpose in similar devices for preparing analytes (see relevant passages cited in the Search Report):

4.1 Document D3 discloses in figure 2 an arrangement with two chambers connected by a channel, plungers for changing the volumes of the chambers and a flow regulation means(3-way valve) in the channel linking the two chambers.

4.2 Document D5 discloses a collapsible chamber connected to a piston chamber via a piercing needle, the flow being controlled between the two chambers by a septum connected to the collapsible chamber, the septum being thus a means of flow

regulation.

Amended Claims

1. A device for contamination free preparation of analyte containing sample solution (P), comprising

a first (2) and second chamber (3), which are connected by a channel (6, 8, 10),

wherein the first chamber (2) has means (4) for reversibly changing its volume, and the second chamber (3) has a reversibly changeable volume,

wherein a connector (7, 9), which is provided with a means of flow regulation, is connected to the channel (6, 8, 10) or one of the chambers (2, 3) for loading of a sample solution into the first (2) or the second chamber (3),

characterized in that

there is provided at least one further chamber (15, 20, 21) being filled with a reactant and sealed, the further chamber being connectable to the channel (6, 8, 10) prior to use.

2. The device of claim 1, wherein the chambers (2, 3) and the channel (6, 8, 10) are designed as a single use device.

3. The device of claims 1 or 2, wherein no means of flow regulation is provided between the first (2) and the second chamber (3).

4. The device of any one of claims 1 to 3, wherein the second chamber is provided with a means for reversibly changing the volume.

5. The device of any one of claims 1 to 4, wherein a first connector (7) is connected either to a first channel (6), which extends from an end of the first chamber (2) opposite the first means (4) for reversibly changing the volume of the first chamber (2) or to the first chamber (2).

6. The device of any one of claims 1 to 5, wherein a second connector (9) is connected to a second channel (8), which extends from an end of the second chamber opposite the second means (5) for reversibly changing the volume or to the second chamber (3).

7. The device of any one of claims 1 to 6, wherein a second channel (8) extends from an end of the second cylinder opposite the second piston (5), wherein the channel (8) is connected to the first channel (6) or the first chamber (2).

8. The device of any one of claims 1 to 7, wherein the further chamber (15, 20, 21) has a reversibly changeable volume, preferably a means for reversibly changing the volume and wherein that chamber(s) is (are) connected with the first (2) and/or second chamber (3) through the first (6) or second channel (8) or through a further channel(s) (17, 24, 25), which is (are) connected to a third channel (10) connecting the first (6) and second channel (8).

9. The device of any one of claims 1 to 8, wherein a means of flow regulation is provided between the first (2) and the second chamber (3) on one side and at least one further chamber(s) (15, 20, 21) on the other side.

10. The device of any one of claims 1 to 9, wherein at least one channel between two of the further chambers (15, 20, 21) has a volume larger than the total compressible volume of the system.

11. The device of any one of claims 1 to 10, wherein at least one of the chambers are conically tapered at the end of the chamber opposite the means (4, 5, 14, 22, 23) for reversibly changing the volume with the opening of the respective channels (6, 8, 17, 24, 25) located at the tip of the conus.

12. The device of any one of claims 1 to 11, wherein the means for changing the volume in the first (2), second (3) and/or further chamber(s) (15, 20, 21) is a piston (4, 5, 14, 22, 23).

13. The device of claim 12, wherein the piston(s) have the shape of the end of the chamber opposite to them or can accommodate this shape.

14. The device of claims 12 or 13, wherein the pistons (4, 5) of the first (2) and/or the second chamber (3) comprise an elastic material, which has or can accommodate the shape of the end of the chamber opposite to them.

15. The device of any one of claims 12 to 14, wherein the pistons (16, 22, 23) in further chambers (15, 20, 21) comprise a reduced elasticity in comparison to the pistons (4, 5) in the first chamber (2) and/or second chamber (3).

16. The device of any one of claims 12 to 15, wherein the pistons (16, 22, 23) are not connected to a piston rod.

17. The device of any one of claims 1 to 16, wherein the chambers (2, 3, 15, 20, 21) have an essentially round cross-section.

18. The device of any one of claims 1 to 17, wherein at least one chamber (2, 3, 15, 20, 21) preferably at least the second

(3) and/or the further chamber(s) (15, 20, 21) are connectible to the channel(s) (8, 17, 24, 25).

19. The device of any one of claims 1 to 18, wherein the axes of the chambers (2, 3, 15, 20, 21) are arranged parallel to each other.

20. The device of any one of claims 1 to 19, wherein in one of the chambers (2, 3, 15, 20, 21), preferably in the second chamber (3) a liquid (L) is provided capable of solubilizing organic substances comprising an analyte and wherein the organic substances are preferably cells.

21. The device of any one of claims 1 to 20, wherein in at least one of the chambers (2, 3, 15, 20, 21) or in at least one of the channel(s) (6, 8, 10, 17, 24, 25) magnetic particles (18) are provided capable of binding the analyte.

22. The device of claim 21, wherein the magnetic particles (18) have a diameter in the range from 50 nm to 50 μm, preferably from 200 nm to 20 μm.

23. The device of any one of claims 1 to 22, wherein in one of the chambers (2, 3, 15, 20, 21), preferably in a further chamber (20), a wash solution (W) is provided.

24. The device of any one of claims 1 to 23, wherein in one of the chambers (2, 3, 15, 20, 21), preferably in a further chamber (21), an elution solution (E) is provided.

25. The device of any one of claims 1 to 24, wherein the connectors (7, 9) are provided with a means of flow regulation, preferably a valve or septum.

26. The device of any one of claims 1 to 25, wherein the chambers (2, 3, 15, 20, 21) are fluid tight against the surrounding when the connector(s) (7, 9) are closed.

27. The device of any one of claims 1 to 26, equipped to accommodate the positioning of a magnet at the end of the chamber(s) (2, 3, 15, 20, 21), preferably the first (2) or the second chamber (3).

28. The device of any one of claims 1 to 27, wherein the device is provided with an enclosure (1) and wherein the enclosure (1) is preferably made of synthetic material.

29. The device of any one of claims 1 to 28 wherein the channels (6, 8, 17, 24, 25) and the connectors (7, 9) are comprised in a base plate (30).

30. The device of claim 29, wherein at least the first chamber, preferably all chambers (2, 3, 15, 20, 21) open up towards the edge of the enclosure (1), so that the means (4, 5, 14, 22, 23) for reversibly changing the volume can be operated from the outside.

31. The device of claims 29 or 30, wherein the enclosure (1) and/or the base plate (30) provided with a means (13) for attaching the device in a corresponding receptacle to allow automatic changing of the volume of at least one chamber (2, 3, 15, 20, 21).

32. A kit of parts comprising a base plate comprising a channel (6, 8, 10), wherein a connector (7, 9), which is provided with a means of flow regulation, is connected to the channel (6, 8, 10) and at least one at least one chamber (15, 20, 21) being filled with a reactant and sealed, the chamber (15, 20, 21) being connectable to the channel (6, 8, 10) prior to use.

33. A method for contamination free preparation of analyte(s) from organic substance comprised in a sample solution (P), using a device according to any one of claims 1 to 31 comprising the following steps:

introducing a predetermined volume of sample solution (P) through a connector (7, 9) into the first (2) or second chamber (3),

interrupting the flow directed through the connector (7, 9),

moving back and forth of the sample solution (P) between the first (2) and the second (3) chamber in such that the sample solution is contacted with material binding or adsorbing the analyte and that the analyte(s) comprised in the sample solution (P) can bind or adsorb to the material, and

dislodging of the analyte(s) through a connector (7, 9).

34. The method of claim 33 comprising the following step: optionally eluting the analyte from said material.

35. The method of claim 33 or 34, wherein the analyte is selected from the group consisting of nucleic acids and polypeptides.

36. The method of one of claims 33 to 35, wherein the material binding or adsorbing the analyte coats at least part of the surface of the chambers (2, 3, 15, 20, 21) and/or channels (6, 8, 17, 24, 25) or particles, in particular magnetic particles comprised within the chambers and/or channels.

37. The method of claim 36, wherein the analyte(s) is (are) dislodged bound to the magnetic particles (18) or separate from the magnetic particles (18).

38. The method of one of claims 33 to 37, wherein the sample solution (P) is moved back and forth by alternately extending and reducing the volume of the first chamber (2).

39. The method of one of claims 33 to 38, wherein the sample solution (P) is moved back and forth by alternately moving the first (4) and the second means (5) for reversibly changing the volume towards the end of the chamber opposite to the means (4, 5).

40. The method of one of the claims 33 to 39, wherein the sample solution (P) is in a further step sonicated and/or mixed with a liquid (L) for solubilization of the organic substance comprised in the sample solution (P).

41. The method of one of the claims 36 to 40, wherein the magnetic particles (18) and the analyte bound thereon are retained in a predetermined region of a chamber (2, 3), preferably the end of the first (2) or second chamber (3) opposite to the means for reversibly changing the volume (4, 5) by generating a magnetic field in the predetermined region of the chamber (2, 3).

42. The method of one of claims 33 to 41, wherein in a further step sample solution (P) depleted of analyte(s) is substantially removed from the chamber(s) (2, 3).

43. The method of claims 33 or 42, wherein a wash solution (W) provided in one further chamber (20) is flown over surface coated with binding material or mixed with the particles, preferably magnetic particles (18).

44. The method of claim 43, wherein the magnetic particles (18) and the analyte bound thereon are retained in a predetermined region of a chamber, preferably the end of the first (2) or second chamber (3) opposite to the means for reversibly

changing the volume (4, 5) by generating a magnetic field in the predetermined region of the chamber (2,3).

45. The method of claims 43 or 44, wherein in a further step wash solution (W) is substantially removed from the chamber(s) (2, 3).

46. The method of one of claims 33 to 45, wherein an elution solution (E) provided in one further chamber (21) flown over surface coated with binding material or mixed with the particles, preferably magnetic particles (18).

47. A method according to claim 46, wherein the magnetic particles (18) are retained in a predetermined region of a chamber, preferably the end of the first (2) or second chamber (3) opposite to the means for reversibly changing the volume (4, 5) by generating a magnetic field in the predetermined region of the chamber.

48. The method of claims 46 or 47, wherein in a further step elution solution (E) comprising the analyte is substantially dislodged from the chamber (2, 3).

49. The method of one of claims 46 to 48, wherein the elution volume is in a range from about 1 to about 100 μl.

50. The method of one of claims 33 to 49, wherein the sample solution (P) depleted of analyte, the liquid (L) for solubilization mixed with the sample solution (P) depleted of analyte, the wash solution (W) and/or the magnetic particles (18) are collected in one of the chambers (2, 3, 15) and are discarded after dislodging, preferably together with the enclosure (1).

51. The method of one of claims 33 to 50, wherein the flow of liquids between two chambers (2, 3, 15, 20, 21) is controlled

by alternately extending and reducing the volume of one chamber (2, 3, 15, 20 ,21), while keeping the volume of all but one of the other chambers (2, 3, 15, 20, 21) constant.

52. The method of one of claims 33 to 50, wherein the flow of liquids between two chambers (2, 3, 15, 20, 21) is controlled by alternately moving one means (4, 5, 14, 22, 23) and a second means (4, 5, 14, 22, 23) for reversibly changing the volume towards the end of the chamber (2, 3, 15, 20, 21) opposite to the means, while keeping the volume of all other chambers (2, 3, 15, 20, 21) constant.

53. A method according to one of the claims 33 to 52, wherein no further liquid except the sample solution (P) is introduced into the system formed by the chambers (2, 3, 15, 20, 21) and the channels (6, 8, 10, 17, 24, 25).